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A comparative study of the leaf epidermis of forty-two native California ferns

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A COMPARATIVE STUDY OF THE LEAF EPIDERMIS OF

FORTY-TWO NATIVE CALIFORNIA FERNS

A THESIS

PRESENTED to

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Master of Science

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by

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Dated 20 July 1967

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Grateful acknowledgment is made to Dr. Walter M. Hewitson, of the University of the Pacific, Dr. S. Conrade Head, of Oregon College, and my family for their encouragement and assistance in the preparation of this paper.

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INTRODUCTION

Fern classification is in a state of flux, the largest area of disagreement being the family Polypodiaceae (Wagner, 1959). This polyphyletic group (Eames, 1936) has been divided by several prominent workers into many families. Ching (1940) recognized thirty-two, Holttum (1947) five, and Copeland (1947) nine families. Munz and Keck (1959) divided the old family Polypodiaceae, as represented by the ferns of California, into five families. The classification of Munz and Keck is the most recent treatment for the native California ferns and is followed in this paper.

Historically, classification of the leptosporangiate ferns has been based on the sorus, but the last half century has seen greater use vegetative characters. Problems of phylogenetic relationships can only be solved by the study of a number of features from all aspects of the plant. The more characters considered and evaluated, the more accurate and valuable are the conclusions.

This line of reasoning has been pursued fruitfully in other plant groups as exemplified by Metcalfe (1960) in his work on the anatomy of the monocotyledons. In this work the microscopical characters of the lamina were taken as being of major importance because of easy availability and the fact that one does not have to have the plant in flower before investigations can proceed.

Another important reason for studying vegetative characteristics is that they are often preserved in the fossil record. Black (1929), Odell (1932), Florin (1951), and Greguss (1961) have studied cutinized superficial leaf epidermis preserved as fossils. The work of Florin has been particularly important in clarifying the phylogeny of the living conifers.

The epidermis of many plant groups has been studied (Faruqi, 1962; Pant and Kidwal, 1964; Pant and Benerji, 1965). The use of epidermal characters has been employed in phylogenetic and taxonomic considerations (Howell, 1945; Prat, 1949; Stebbins and Kush, 1961; Barbrov, 1962; Baranova, 1962; Argus, 1965; Stace, 1965, 1966). Little work has been done on the epidermis of the ferns (Kondo, 1929, Kondo and Toda, 1956; Wylie, 1948; Allsopp, 1955; Maroti, 1958, 1961). Several workers have considered the fern allies (Gulyas, 1961; Pant and Mehra, 1964), but a survey of the literature reveals that the ferns have not received the intensive treatment of other groups.

The question of the value of the epidermis in the study of the ferns still remains an unsolved problem. Therefore, it is the intention of this paper to explore the possibility of using epidermal characters as a basis for establishing patterns of morphological and taxonomic significance. Because of their easy availability, the native ferns of California were used for this study.

MATERIALS AND METHODS

The forty-two ferns studied are listed by species in Table I. The source of the collection and the collector's name and number are given. Voucher specimens are filed in the Herbarium of the University of the Pacific.

To be as consistent as possible, an effort was made to utilize pinnules from the mid-portion of the frond. This worked very well except for the genus *Pellaea* whose margins roll in greatly upon drying and do not expand upon rehydration. For this reason the abaxial epidermis was difficult to observe. The first fronds put out by the genus *Pellaea* are generally sterile, and these were utilized in place of the fertile frond.

In cases of extreme vestiture, as in the genus *Cheilanthes*, hairs and scales were plucked with forceps from the rehydrated pinnae and mounted in Diaphane for observation.

Pinnae were cleared of chlorophyll using the method of Foster (1934). Staining was done using the tannic acid-iron chloride method also of Foster (1934). Herbarium specimens were first rehydrated in hot water to which a few granules of detergent had been added.

The following steps were then taken:

1. Remove water and boil in 95% alcohol.
2. Wash with tap water several times.
3. Transfer to 2.5% NAOH and leave overnight.
4. Rinse in tap water.
5. Place in 50% solution of Chlorox and water until clear.
6. Rinse in tap water.
7. Place in 50% alcohol overnight.
8. Place in 70% alcohol for 15-20 minutes.
9. Stain 4-5 minutes in iron chloride (5% solution in 70% alcohol).
10. Stain a few seconds in tannic acid, (5% solution in 70% alcohol).
11. Rinse in 70% alcohol.
12. Place in 95% alcohol 15-20 minutes.
13. Mount in Diaphane.

Slides were examined and photographed at 430 diameters. The drawings were made from the negative using a photographic enlarger.

TABLE I
SPECIES OF FERNS STUDIED

Species	Plate and Figure Number	Source
Botrychium multifidum (Gmel.) Rupr. ssp. *silaefolium* (Presl.) Clausen.	Pl. I, Fig. 1	Placer County: 2 miles southeast of Soda Springs; Hebert 177.
Marsilea vestita Hook. and Grev.	Pl. I, Fig. 2	University of the Pacific Herbarium number 33.
Pteridium aquilinum (L.) Kuhn var *lanuginosum* (Bong.) Fern.	Pl. I, Fig. 3	Amador County: 1 mile east of Pioneer; Hebert 96.
Cheilanthes jonesii (Maxon) Munz.	Pl. I, Fig. 4	San Bernardino County: 1 mile north of Mountain Pass, Clark Mountains; Hebert 169.
Cheilanthes newberryi (D. C. Eat.)	Pl. I, Fig. 5	San Diego County: Highway 395 between Temecula and Fallbrook; Harriet Baker 546.
Cheilanthes parryi	Pl. II, Fig. 6	San Bernardino County: Mitchell's Cavern's Trail, Providence Mountains; Hebert 147.

TABLE I (continued)

Species	Plate and Figure Number	Source
Cheilanthes feei T. Moore.	Pl. II, Fig. 7	San Bernardino County: Freeman Canyon, New York Mountains; Hebert 146
Cheilanthes gracillima D. C. Eat.	Pl. II, Fig. 8	Sierra County: 1 mile north of Sierra City in the Sierra Buttes; Hebert 171.
Cheilanthes clevelandii D. C. Eat.	Pl. II, Fig. 9	San Diego County: Highway 395; Harriet Baker 554.
Cheilanthes covillei Maxon.	Pl. II, Fig. 10	Lake County: Bartlett Springs Road, 5 miles north of Clear Lake; Hebert 161.
Cheilanthes wootonii Maxon.	Pl. III, Fig. 11	San Bernardino County: Freeman Canyon, New York Mountains; Hebert 172.
Aspidotis californica Nutt.	Pl. III, Fig. 12	Stanislaus County: Below Knights Ferry Bridge; Hebert 173.
Pellaea compacta (Davenp.) Maxon.	Pl. III, Fig. 13	San Bernardino County: Caruther's Canyon, New York Mountains; Hebert 174.

TABLE I (continued)

Species	Plate and Figure Number	Source
Pellaea brachyptera (T. Moore) Baker	Pl. III, Fig. 14	Sierra County: 1 mile north of Sierra City, foot of Sierra Buttes; Hebert 175.
Pellaea mucronata (D. C. Eat.) Eat.	Pl. III, Fig. 15	Calaveras County: Milton Copperopolis Road, Rock Springs Mountain Road, Rock Springs Creek; Hebert 57.
Pellaea andromedaefolia (Kaulf.) Fee.	Pl. IV, Fig. 16	Napa County: York Creek, Spring Mountain Road from St. Helena to Santa Rosa; Hebert 115.
Pellaea bridgesi Hook	Pl. IV, Fig. 17	Tuolumne County: East end Pinecrest Lake (Strawberry Lake); Hebert 114.
Pellaea breweri D. C. Eat.	Pl. IV, Fig. 18	Tuolumne County: Sonora Pass, Chipmunk Flat, Deadman's Creek; Hebert 166.
Cryptogramma acrostichoides R. Br.	Pl. IV, Fig. 19	Tuolumne County: Highway 108, 1 mile east of Douglas Resort; Hebert 112.

TABLE I (continued)

Species	Plate and Figure Number	Source
Onychium densum Brack.	Pl. IV, Fig. 20	Sierra County: Upper Sardine Lake, North of Sierra Buttes; Hebert 176.
Pityrogramma triangularis (Kaulf.) Maxon	Pl. V, Fig. 21	Calaveras County: 50 yards south of bridge, North Fork of the Mokelumne River between Pioneer and West Point; Hebert 100.
Pityrogramma triangularis var. *vicosa* (Nutt. ex D. C. Eat.) Weath.	Pl. V, Fig. 22	San Diego County: Highway 395 between Temecula and Fallbrook; Harriet Baker 566.
Pityrogramma triangularis var. *pallida* Weath.	Pl. V, Fig. 23	Calaveras County: Milton-Copperopolis Road, Rock Creek; Hebert 157.
Adiantum pedatum L. var. *aleuticum* Rupr.	Pl. VII, Fig. 34	Napa County: York Creek Spring Mountain Road from St. Helena to Santa Rosa; Hebert 49.
Adiantum jordanii C. Muell.	Pl. VII, Fig. 35	Calaveras County: Milton-Copperopolis Road, Rock Creek; Hebert 34.

TABLE I (continued)

Species	Plate and Figure Number	Source
Adiantum capillus veneris L.	Pl. VIII, Fig. 36	Santa Cruz County: Santa Cruz Mountains, near Big Basin State Park; Hebert 34.
Woodsia scopulina D. C.	Pl. VI, Fig. 26	Siskiyou County: 4 miles north of Weed on Highway 97; Hebert 150.
Polystichum munitum (Kaulf.) Presl.	Pl. VI, Fig. 27	Marin County: Fairfax Bolinas Road, Alpine Dam; Hebert 36
Polystichum munitum imbricans (D. C. Eat.) Maxon.	Pl. VI, Fig. 28	Tuolumne County: East end of Pinecrest Lake, Highway 108; Hebert 111.
Polystichum californicum (D. C. Eat.) Underw.	Pl. VI, Fig. 29	Mendocino County: Talmadge, water supply to Mendocino State Hospital; Hebert 151.
Polystichum dudleyi Maxon.	Pl. VI, Fig. 30	Marin County: Fairfax Bolinas Road, Alpine Dam; Hebert 21.
Dryopteris dilatata (Hoffm.) Gray.	Pl. VIII, Fig. 31	Del Norte County: 3 miles east of Klamath on road to Klamath Glen; Hebert 156.

TABLE I (continued)

Species	Plate and Figure Number	Source
Dryopteris arguta (Kaulf.) Watt.	Pl. VII, Fig. 32	Mendocino County: Road from Highway 101 to Dos Rios on the Eel River; Hebert 149.
Cystopteris fragilis (L.) Bernh.	Pl. VII, Fig. 33	Placer County: Serena Creek, Ice Lakes, 2 miles south-east of Soda Springs; Hebert 9.
Athyrium Filix-femina (L.) Roth var. *californicum* Butters.	Pl. V, Fig. 24	Placer County: Serena Creek, Ice Lakes, 2 miles east of Soda Springs; Hebert 12.
Athyrium filix-femina var. *Sitchense* Rupr.	Pl. V, Fig. 25	Marin County: Fairfax to Bolinas Road, near Alpine Dam; Hebert 164.
Blechnum spicant (L.) With.	Pl. VII, Fig. 37	Mendocino County: Highway 1, 3 miles south of Rockport; Hebert 16.
Woodwardia fimbriata Sm.	Pl. VIII, Fig. 38	Calaveras County: Milton-Copperopolis Road, Rock Creek; Hebert 75.
Asplenium vespertinum Maxon.	Pl. VII, Fig. 39	University of California Herbarium number 811,349.

TABLE I (continued)

Species	Plate and Figure Number	Source
Polypodium scouleri Hook. and Grev.	Pl. VIII, Fig. 40	Marin County: Elephant Rocks, Dillon Beach Road; Hebert 44.
Polypodium californicum Kaulf.	Pl. IX, Fig. 41	Marin County: Fairfax Bolinas Road, Oak-madrone forest; Hebert 19.
Polypodium glycyrrhiza D. C. Eat.	Pl. IX, Fig. 42	Mendocino County: Highway 1, 3 miles south of Rockport; Hebert 148.

OBSERVATIONS

The nomenclature of Maróti (1959) was followed in the observations. He distinguished three general fern types: (A) the Ophioglossaceae type, (B) the Marsileaceae type, and (C) the Polypodiaceae type. In order to classify ferns under these types, Maróti recognized four types of arrangements of the cells around the stomata. These are:

(A) Acyclic: guard cells surrounded by three to six epidermal cells. Botrychium multifidum (Plate I, Fig. 1, B) is a good example of this type.

(B) Amphicyclic: guard cells surrounded on all sides by curved, occluding cells. This type is represented by Angiopteris, not native to California.

(C) Unicyclic: guard cells located in the center of one epidermal cell. This type is represented by Anemia, also not native to California.

(D) Monocyclic: the guard cells partially surrounded by one central polar cell and the remainder of the guard cells surrounded by two to three epidermal cells. The epidermal cell surrounding the guard cell pole on the side of the rachis is referred to as the central polar cell. This cell is peculiar to one stomata and is not shared by an adjacent stomata. Polypodium scouleri (Plate VIII, Fig. 40, B) illustrates this type.

In the following discussion the word "epidermis" is used to refer to the epidermal ground cells and "guard cells" is used to refer to the two cells guarding the stoma. This is done with the recognition that guard cells as well as ground epidermis are protodermal in origin.

<u>Botrychium</u> Sw.
Plate I, Fig. 1

The leaf is amphistomatic. Stomata are acyclic with the guard cells surrounded by five, six, or seven epidermal cells. Twin stomata were reported by Maróti (1961) for *Botrychium lunaria* (L.) Sw., but none were seen by the author in the California species, *Botrychium multifidum*. The longitudinal axis of the guard cells runs parallel with the veins.

The cells of the epidermis are anisodiametric and elongated with four to five corners. No elongated epidermal cells were observed over the veins on either the adaxial or abaxial surfaces of the leaf.

Marsilea L.
Plate I, Fig. 2

The leaf is amphistomatic when extending above the surface of the water, but is epistomatic when the leaf is floating (Allsopp, 1955). The specimens in this study were floating and epistomatic. The stomata are acyclic, each one surrounded by three to four epidermal cells.

Epidermal cells of the abaxial surface are more irregular in outline than the adaxial surfaces. Cell walls of the abaxial epidermal cells are elongated and irregular with some straight-walled and some lobed. No differences in the shapes of the epidermal cells overlaying the veins were noted.

Pteridium Scop.
Plate I, Fig. 3

The leaf is hypostomatic. Stomata are monocyclic and are surrounded by two or three epidermal cells in addition to the central polar cell. The long axis of the guard cells runs parallel to the veins.

Radial walls of the epidermal cells are irregularly convoluted and deeply lobed. The epidermal cells over the veins of the lower surface are elongated and straight-walled.

Abaxial and adaxial surfaces are moderately covered with one, two, or three-celled hairs found on the veins as well as on the interveinal spaces (Plate X, Fig. 9).

Cheilanthes Sw.
Plate I, Figs. 4, 5
Plate II, Figs. 6, 7, 8, 9, 10
Plate III, Fig. 11

The leaves are hypostomatic. Stomata are acyclic; each guard cell is surrounded by four to five epidermal cells. *Cheilanthes clevelandii*, *Cheilanthes covillei*, *Cheilanthes feei*, and *Cheilanthes jonesii* show some tendency toward being monocyclic, but the majority of the guard cells are irregularly surrounded by epidermal ground cells with no one occupying the prominent position of the central polar cell. The long axis of the guard cells runs parallel to the veins.

Upper epidermal cells exhibit the characteristic wavy, jig-saw puzzle-like appearance, which is not remarkable except for *Cheilanthes newberryi* and *Cheilanthes feei* in which the cells are smaller. The adaxial epidermis is elongated with straight walls over the veins; the cells have tapering ends which merge with the adjacent cells.

PLATE I

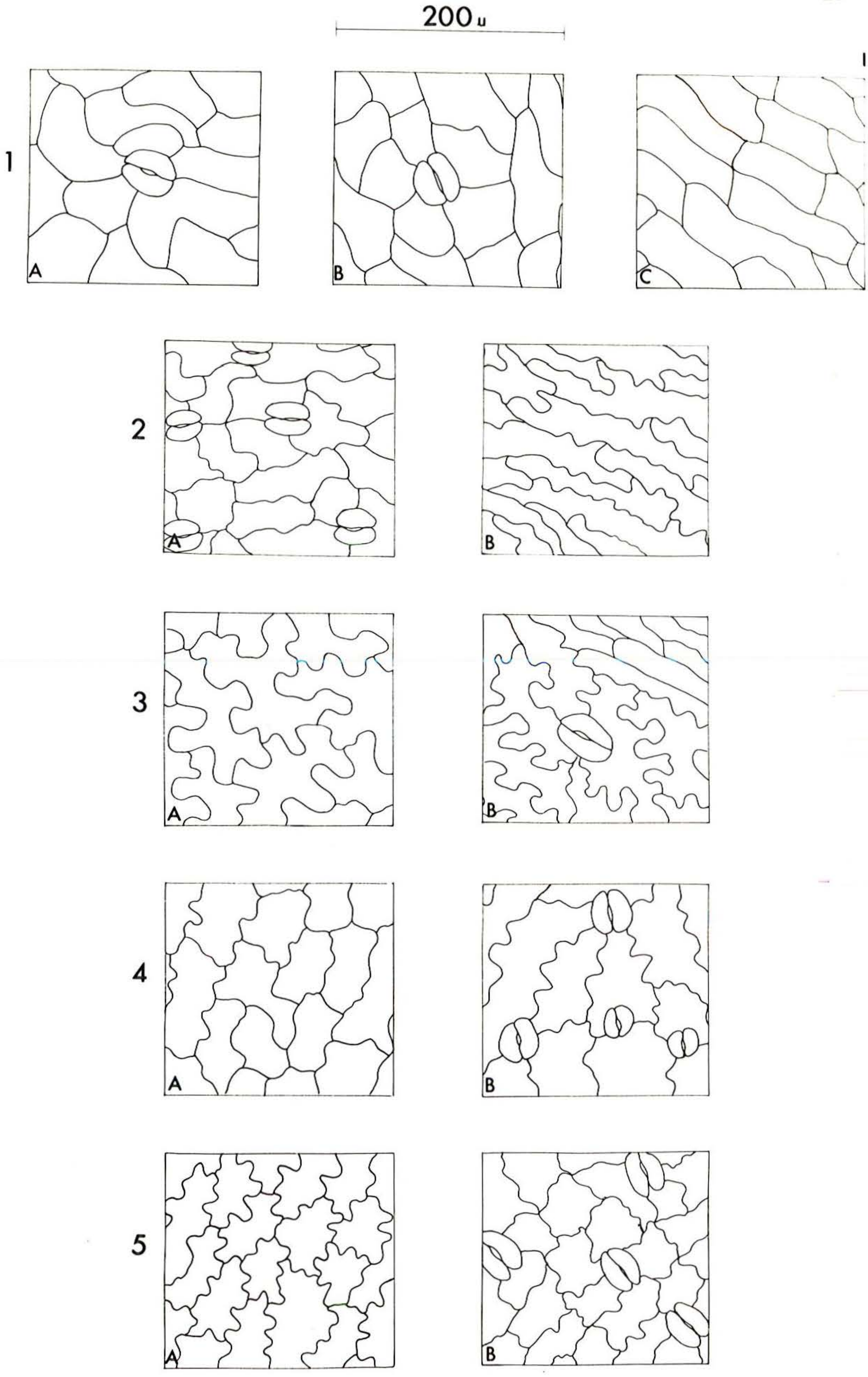
Figure 1. Botrychium multifidum (A) upper epidermis, (B) lower epidermis, (C) lower epidermis between stomata.

Figure 2. Marsilea vestita (A) upper epidermis, (B) lower epidermis.

Figure 3. Pteridium aquilinum (A) upper epidermis, (B) lower epidermis adjacent to a vein.

Figure 4. Cheilanthes jonesii (A) upper epidermis, (B) lower epidermis.

Figure 5. Cheilanthes newberryi (A) upper epidermis, (B) lower epidermis.


200 µ
1
A
B
C
2
A
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3
A
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4
A
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5
A
B

PLATE II

Figure 6. Cheilanthes parryi (A) upper epidermis, (B) lower epidermis.

Figure 7. Cheilanthes feei (A) upper epidermis, (B) lower epidermis.

Figure 8. Cheilanthes gracillima (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

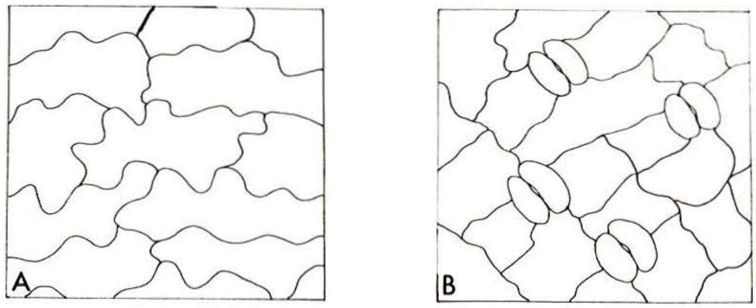
Figure 9. Cheilanthes clevelandii (A) upper epidermis, (B) lower epidermis.

Figure 10. Cheilanthes covillei (A) upper epidermis, (B) lower epidermis.

II

200μ

6



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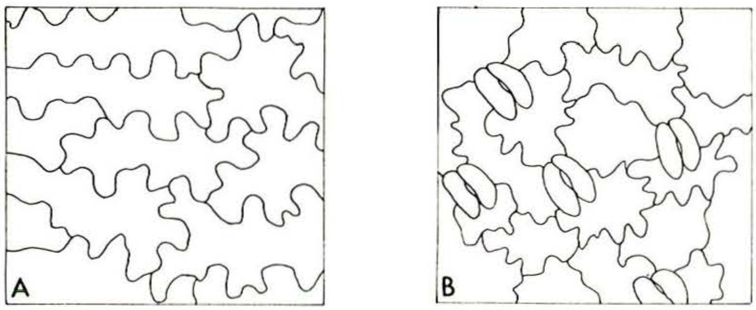

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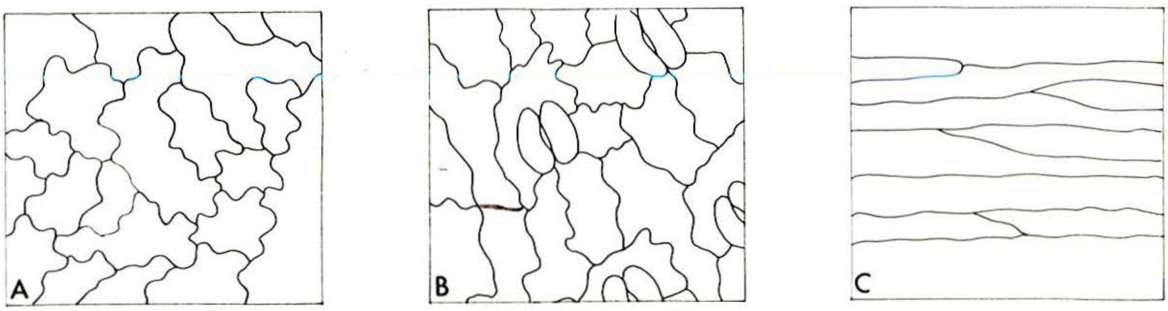

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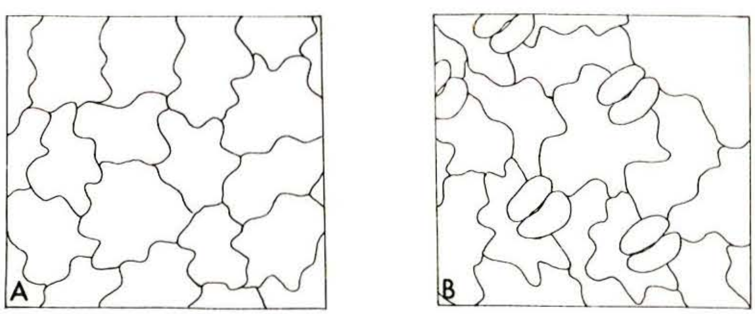

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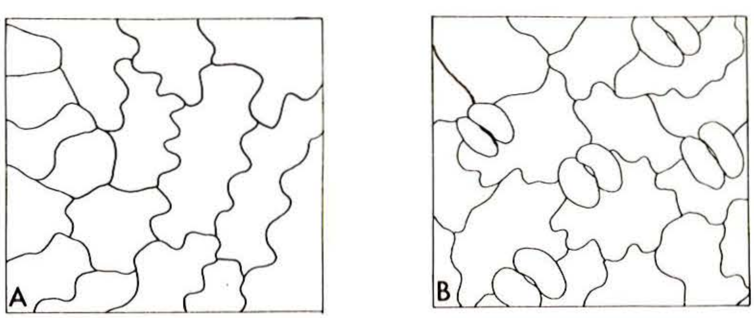


The long axis of the epidermal cells on both abaxial and adaxial surfaces parallels the veins.

Scales and hairs are widespread in this genus, probably representing an adaptation to extremely xerophytic conditions (Plate X, Figs. 10, 11, 14, 16, 17).

<u>Aspidotis</u> Nutt. ex Copel.
Plate III, Fig. 12

The leaf is hypostomatic. Stomata are monocyclic, surrounded by two or three epidermal cells.

Epidermal cells of the adaxial surface are extremly elongated, narrow, and do not possess any of the convolutions which appear in ferns generally. They resemble the cells which overlay the veins in other ferns. The ends are tapering, rounded, and merge with the side of the walls of the neighboring cells. Radial walls of the abaxial epidermal cells are regularly wavy without deep undulations and are also elongated parallel to the veins. Cells over the veins are elongated, wavy, and devoid of stomata.

Scattered over the surface between the veins are numerous two-celled glands (Plate IX, Fig. 4). The word gland is used in the general sense of minute hairs comprising one to several cells (Wagner, 1952). No glands were observed on the lower surface.

Pellaea Link.
Plate III, Figs. 13, 14, 15
Plate IV, Figs. 16, 17, 18

The leaf is hypostomatic. Stomata are monocyclic for the most part although Pellaea compacta, Pellaea breweri, and Pellaea andromaedofolia show a tendency to be acyclic. Guard cells are surrounded by three to four epidermal cells. The central polar cell is less distinct in those species which possess it. This genus seems to present a transitional situation in which there is a graduation from acyclic to monocyclic.

The abaxial epidermal cells over the veins are elongated with straightened walls in all species. The adaxial epidermal cells are deeply lobed with the characteristic jig-saw arrangement.

Cryptogramma R. Br. in Richards.
Plate IV, Fig. 19

The leaf is hypostomatic; long axes of the guard cells run parallel to the veins. Stomata are acyclic or monocyclic, both types being present.

The cells of the adaxial surface present the typical lobed appearance. There is some elongation of the cells over the veins on the adaxial surface, but it is not so marked as over the vein on the abaxial surface. The long axis of these cells runs parallel to the veins.

An occasional three or four-celled gland is present arising from the abaxial epidermal cells over the veins (Plate IX, Fig. 5).

Onychium Kaulf.
Plate IV, Fig. 20

The leaf is hypostomatic; the long axis of the guard cells runs parallel to the veins. Stomata vary from monocyclic to acyclic, they are intermediate in this respect.

The epidermal cells over the veins of the abaxial surface are longated with straightened walls and are devoid of stomata. Epidermal cells surrounding the stomata have wavy radial walls.

PLATE III

Figure 11. Cheilanthes wootonii (A) upper epidermis, (B) lower epidermis.

Figure 12. Aspidotis californica (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

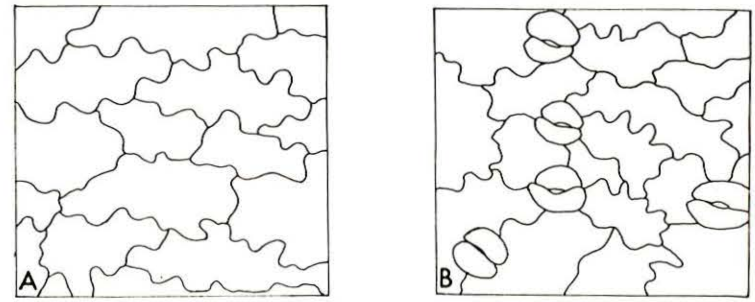
Figure 13. Pellaea compacta (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 14. Pellaea brachyptera (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 15. Pellaea mucronata (A) upper epidermis, (B) lower epidermis.

200 µ

11



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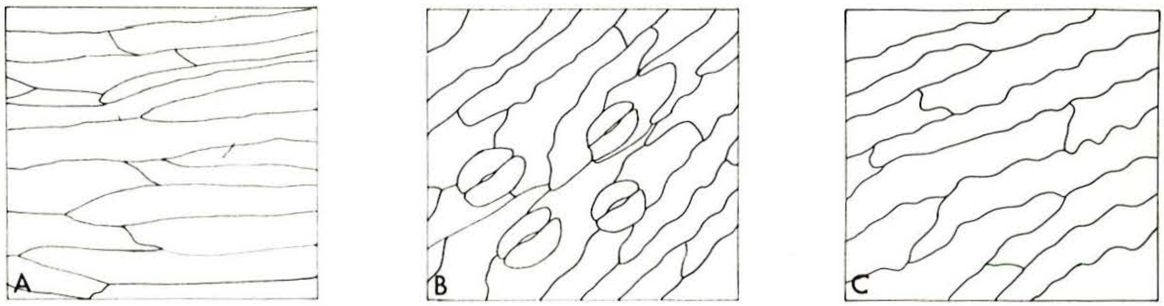

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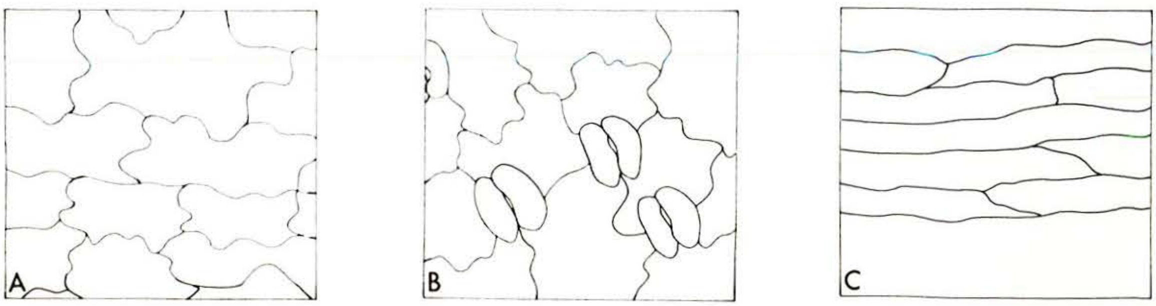

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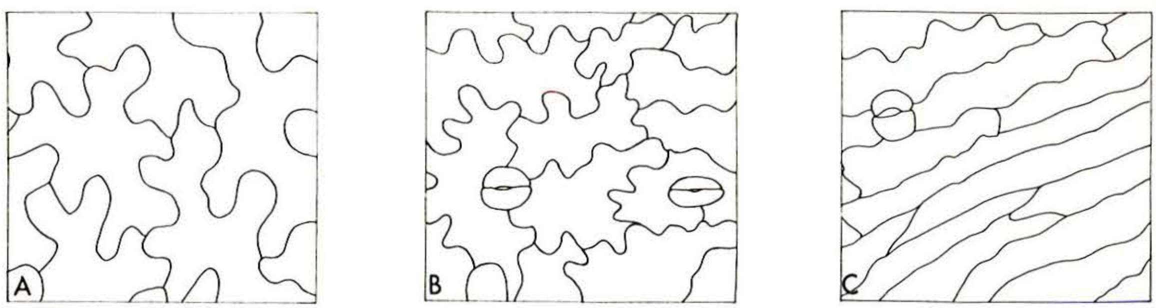

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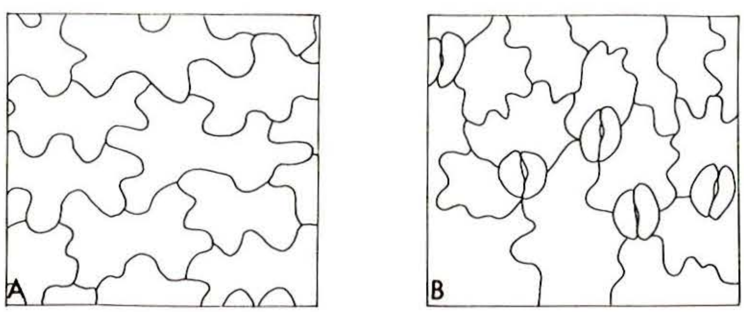


PLATE IV

Figure 16. Pellaea andromedaefolia (A) upper epidermis, (B) lower epidermis.

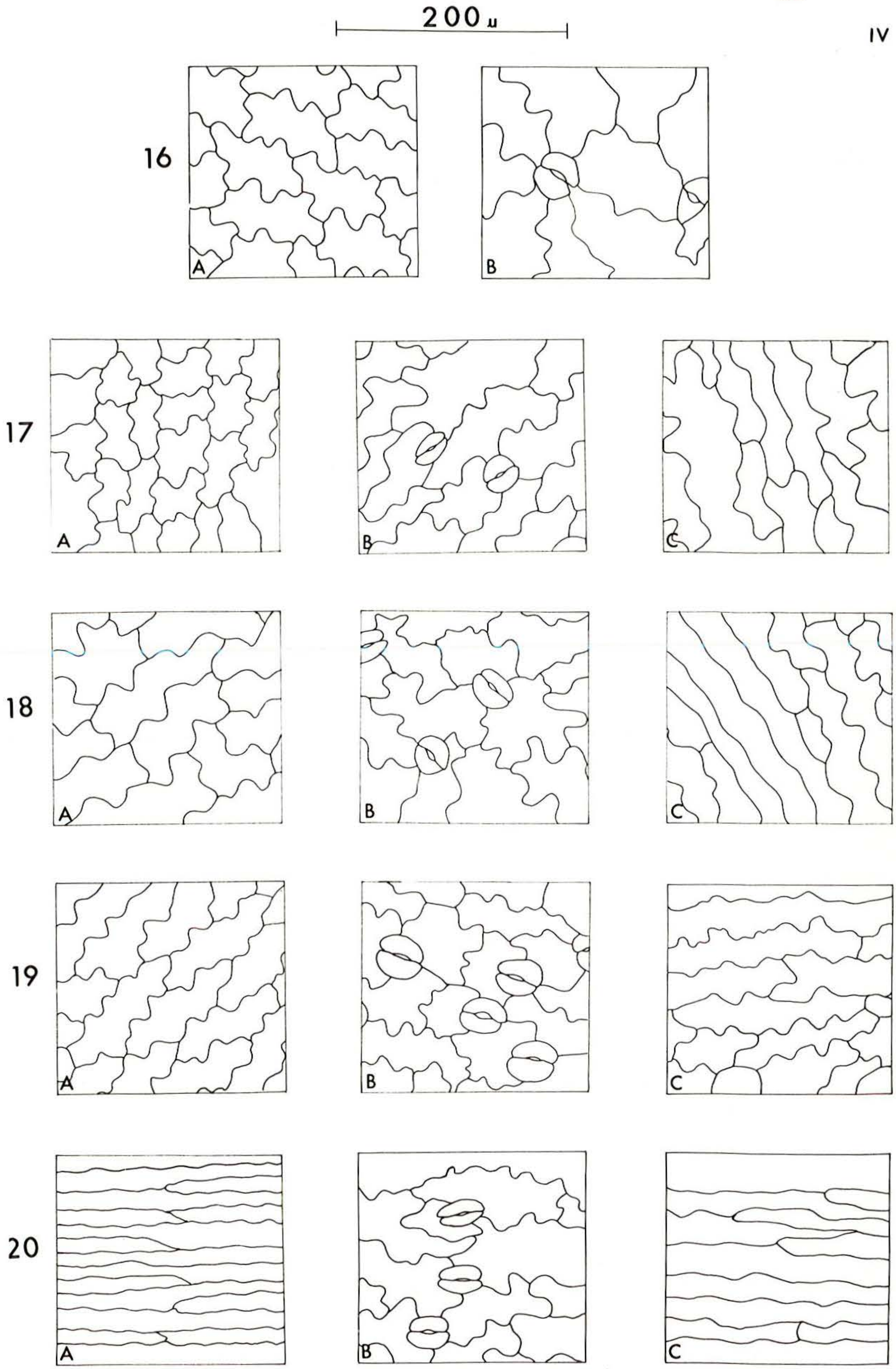
Figure 17. Pellaea bridgesii (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 18. Pellaea breweri (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 19. Cryptogramma acrostichoides (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 20. Onychium densum (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

IV



The cells of the adaxial surface are extremely elongated with straight walls and tapering ends, a complete departure from the usual state of affairs in the family Pteridaceae except for the genus Aspidotis californica. Aspidotis californica possesses elongated adaxial epidermal cells, but these cells are larger than the adaxial cells of Onychium densum. These elongated adaxial cells resemble the cells over the abaxial surface of the veins but are narrower. These two genera were removed from the genus Cheilanthes by Munz and Keck (1959) and placed in the two genera Onychium and Aspidotis.

Pityrogramma Link.
Plate V, Figs. 21, 22, 23

The leaves are hypostomatic; stomata are monocyclic and surrounded usually by four irregularly lobed epidermal cells. Long axes of the guard cells are parallel to the veins.

Epidermal cells of the adaxial surface are anisodiametric with radial wavy walls. The long axis is oriented parallel to the adjacent vein. There is no elongation of the cells over the lesser upper veins, but there is elongation over the major veins. Epidermal cells with regular, wavy cell walls are elongated over the lower veins.

Glands were visible at the leaf edge of *Pityrogramma triangularis* var. *viscosa* (Plate IX, Fig. 3), but none were observed on *Pityrogramma triangularis* or *Pityrogramma triangularis* var. *pallida*.

Athyrium Roth.
Plate V, Figs. 24, 25

The adaxial epidermal cells are very deeply lobed. There are six to seven deep lobes per cell with rounded "teeth". The long axis of these cells runs parallel to the veins. Cells over the veins of the lower surface are elongated.

The leaves are hypostomatic; the axis of the guard cells is parallel to the adjacent vein. The stomata are monocyclic, and the central polar cell is composed of deeply rounded lobed walls. These lobes appear to have cross arms and a squared shape which resembles the letter "T" (Maróti, 1958; Plate V, Fig 24, B; Plate V, Fig. 25, B).

PLATE V

Figure 21. Pityrogramma triangularis (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 22. Pityrogramma triangularis var. viscosa (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 23. Pityrogramma triangularis var. pallida (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 24. Athyrium filix-femina (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 25. Athyrium filix-femina var. sitchense (A) upper epidermis, (B) lower epidermis.

200 µ

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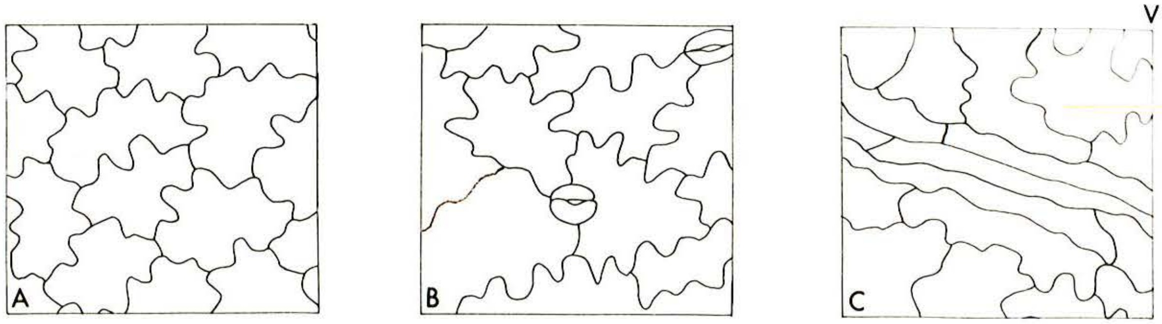

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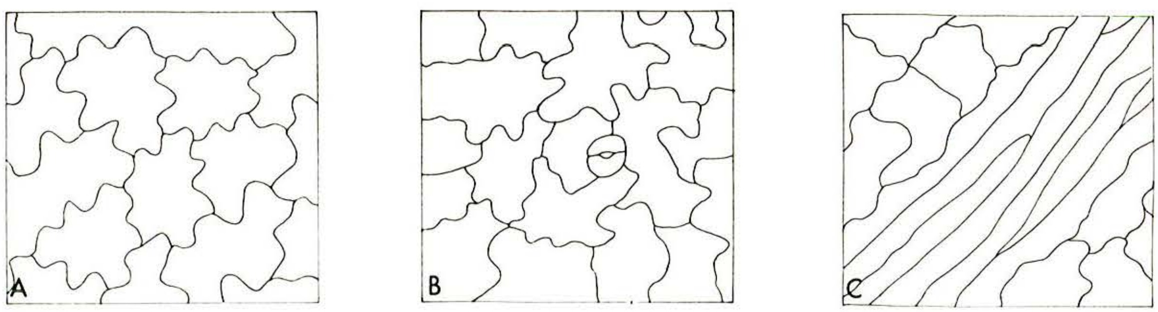

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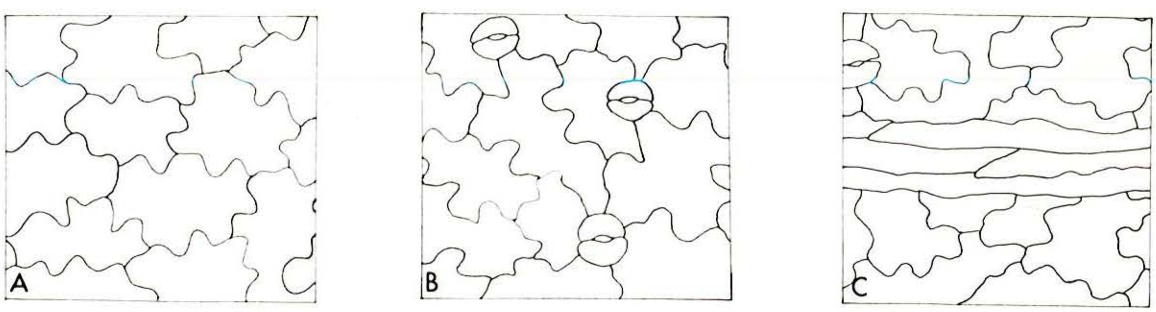

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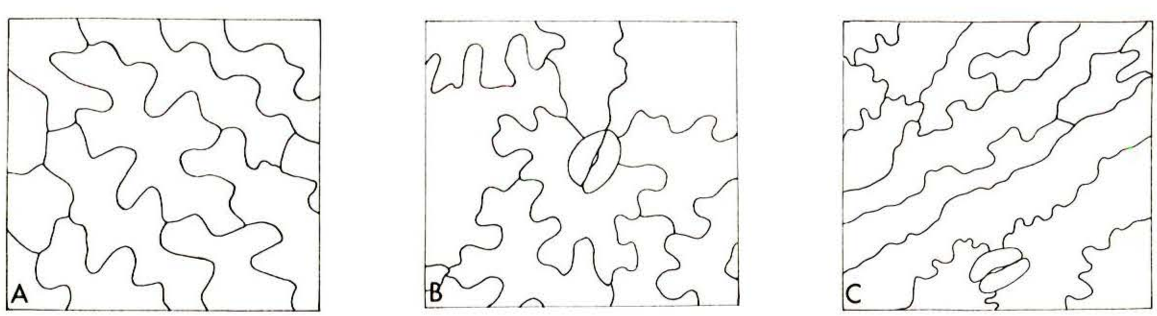

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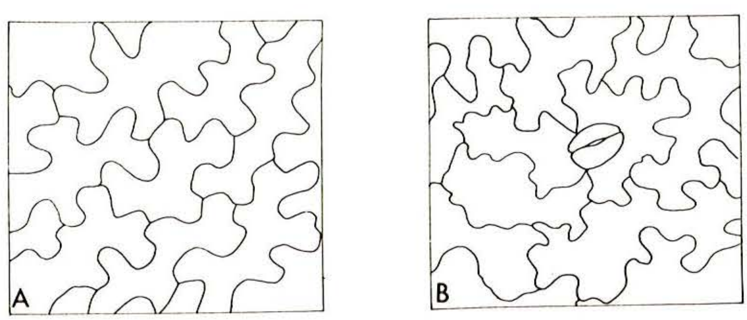


Woodsia R. Br
Plate VI, Fig. 26

The leaf is hypostomatic; the long axis of the guard cells runs parallel to the veins. Stomata are monocyclic; the lobes of the central polar cell are rounded and irregularly indented.

The adaxial epidermis is also deeply lobed; the long axes of the cells run parallel to the veins. The epidermal cells are only slightly elongated over the veins.

Both surfaces of the leaf are covered with numerous three to four-celled hairs scattered between the veins (Plate X, Fig. 12).

Polystichum Roth.
Plate VI, Figs. 27, 28, 29, 30

The leaves are hypostomatic. Stomata are monocyclic and surrounded by three to four epidermal cells. The long axes of the guard cells are parallel to the veins. The central polar cell is regularly lobed with rounded "teeth." *Polystichum dudleyi* and *Polystichum californicum* appear to have acyclic stomata. The upper epidermis of *Polystichum dudleyi* is not so deeply lobed as *Polystichum munitum* and *Polystichum munitum* var. *imbricans*.

The epidermal cells are elongated over the abaxial and adaxial veins and are devoid of stomata, but still retain the lobed walls.

PLATE VI

Figure 26. Woodsia scopulina (A) upper epidermis, (B) lower epidermis.

Figure 27. Polystichum munitum (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

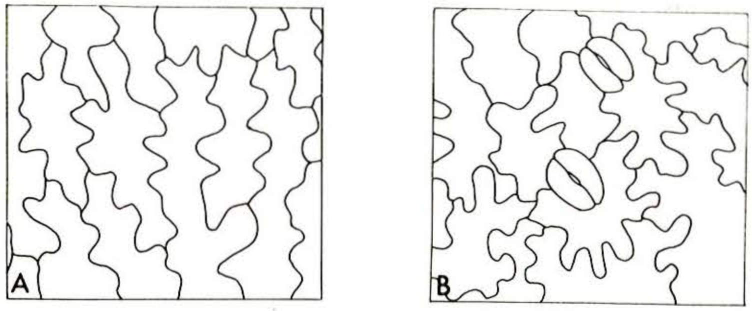
Figure 28. Polystichum munitum var. imbricans (A) upper epidermis, (B) lower epidermis, (C) upper epidermis over a vein.

Figure 29. Polystichum californicum (A) upper epidermis, (B) lower epidermis, (C) upper epidermis over a vein.

Figure 30. Polystichum dudleyi (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

VI

200 u

26


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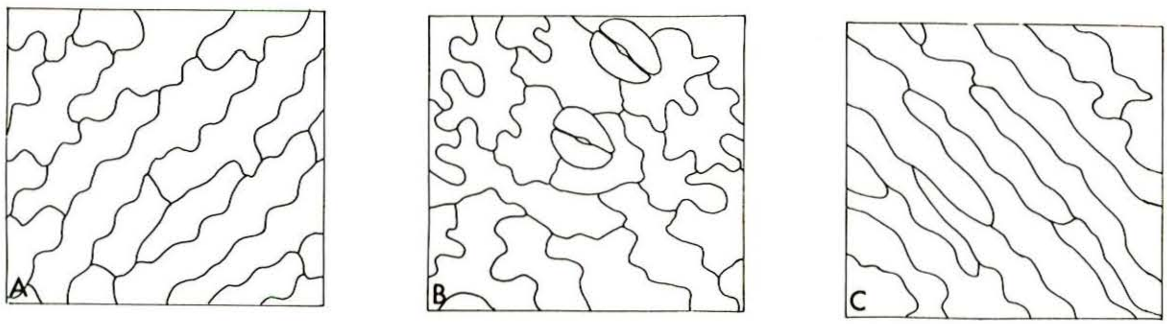

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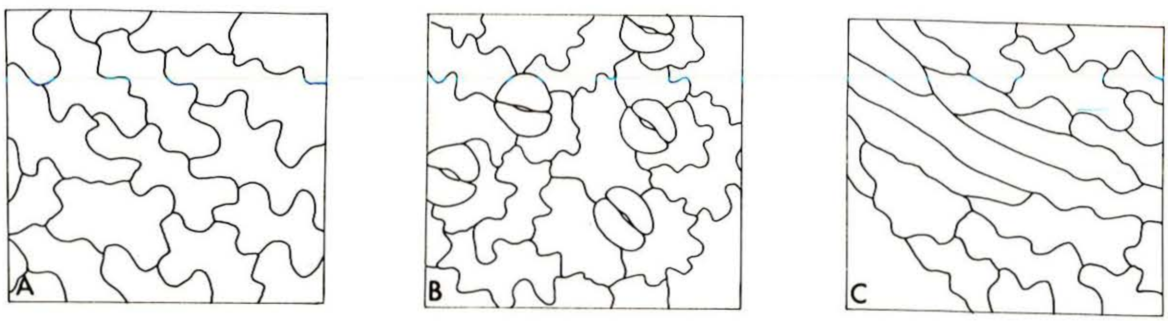

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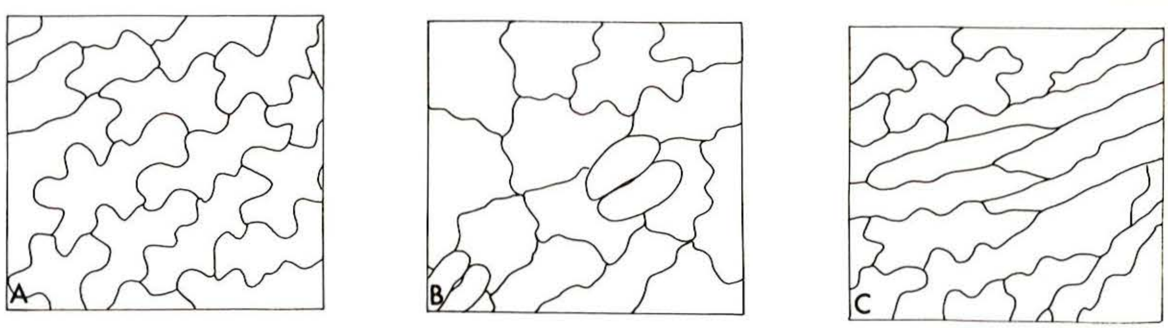

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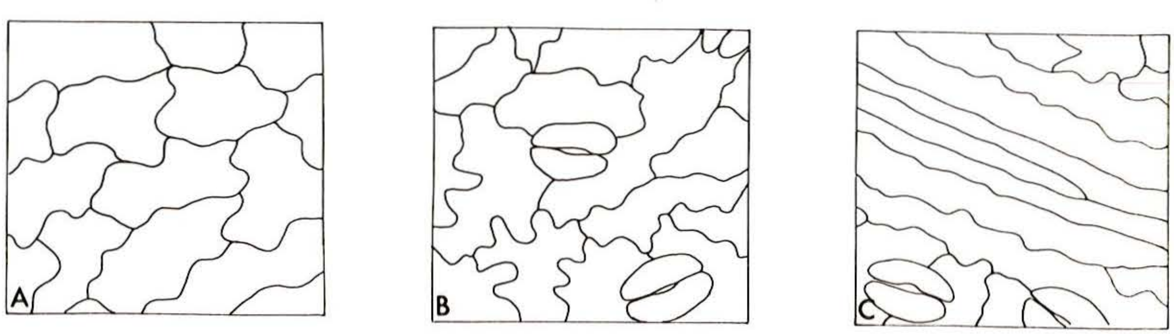


Scattered scales were seen arising from the cells over the veins in Polystichum munitum (Plate X, Fig. 15), but none were observed in Polystichum munitum var. imbricans or the other two species. This may be one additional way of distinguishing Polystichum munitum from Polystichum munitum var. imbricans. The scales of Polystichum dudleyi have a small two-celled stalked scale, unique among the ferns examined (Plate X, Fig. 13).

Dryopteris Adans.
Plate VII, Figs. 31, 32

The leaves are hypostomatic. Stomata are monocyclic, but the central polar cell is not so regularly lobed as is seen in Athyrium and Polystichum. Neither are they as deeply indented in the lobing. The stomata are surrounded by two to three other epidermal cells besides the central polar cell.

The cells over the veins are elongated in Dryopteris dilatata, but still retain the lobed wavy walls. No stomata were found over the veins.

The upper epidermal cells are more deeply lobed than the lower epidermal cells; they are longer than broad with their long axes parallel to the veins. There was no elongation over the veins on the upper epidermis.

Cystopteris Bernh.
Plate VII, Fig. 33

The leaves are hypostomatic. Stomata are monocyclic. The central polar cell is deeply lobed with seven or more rounded lobes. Two or more epidermal cells also surround the guard cells.

The adaxial epidermal cell walls are reminiscent of pieces in a jig-saw puzzle with their lobed walls. The cells over the abaxial veins are elongated with straight walls.

No glands or hairs were observed although these were reported by Blasdell (1936). Perhaps these are present in young leaves but are absent at maturity. According to Wagner (1952), careful examination usually reveals a few remaining hairs on mature epidermal surfaces just as it does in most ferns described as "glabrous" or "non-glandular."

PLATE VII

Figure 31. Dryopteris dilatata (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 32. Dryopteris arguta (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

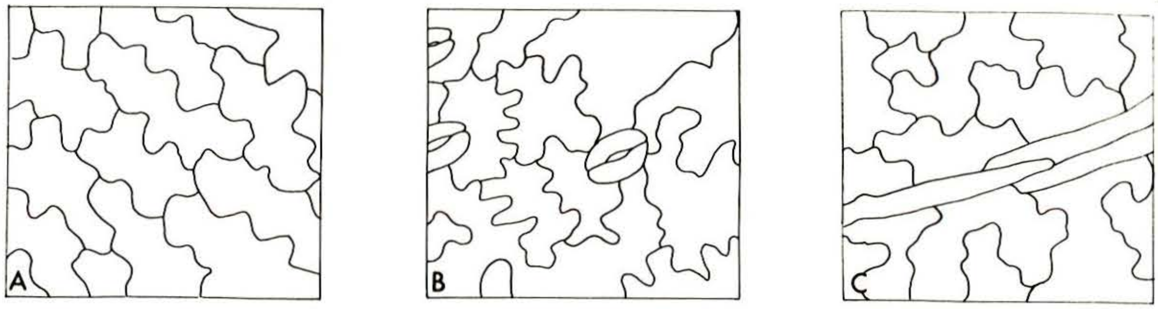
Figure 33. Cystopteris fragilis (A) upper epidermis, (B) lower epidermis.

Figure 34. Adiantum pedatum var. aleuticum (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 35. Adiantum jordani (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

200 µ

31



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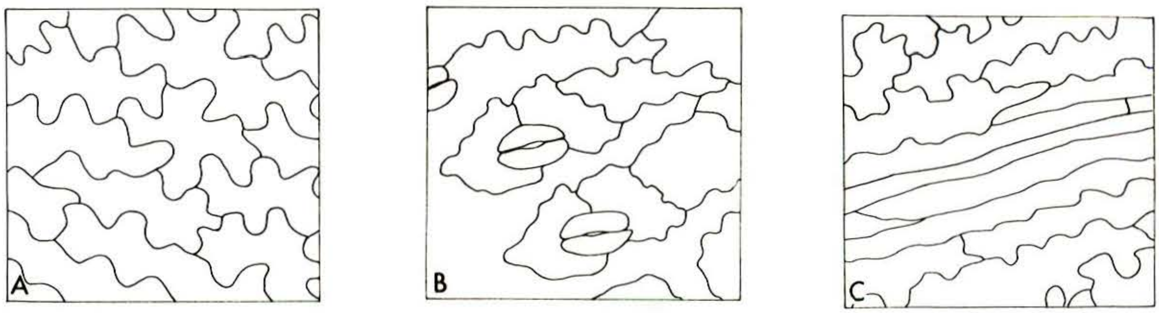

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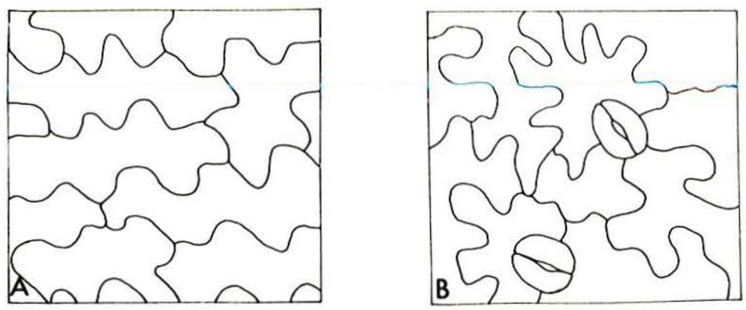

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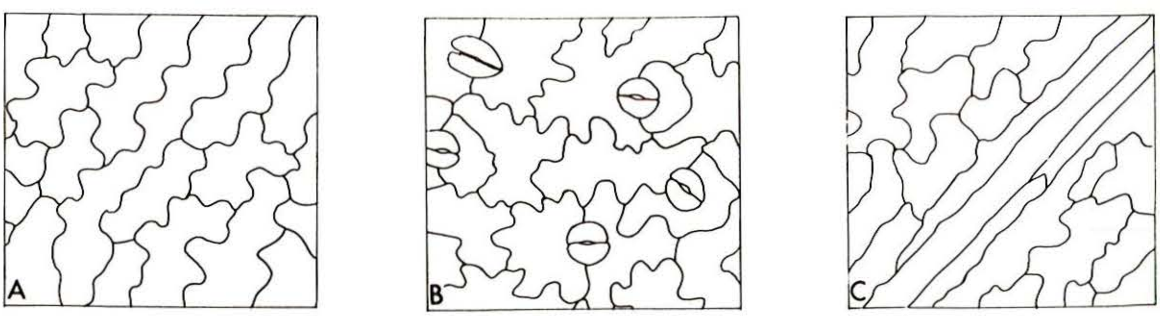

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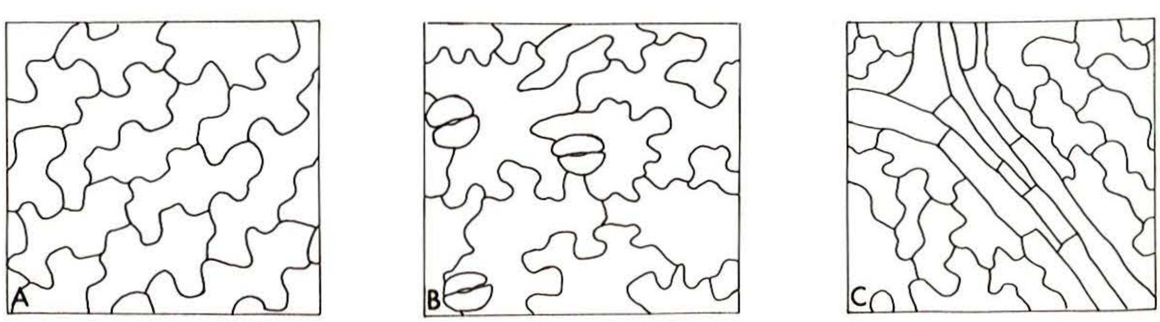


Adiantum L.
Plate VII, Figs. 34, 35
Plate VIII, Fig. 36

The leaves are hypostomatic. Stomata are monocyclic except for Adiantum capillus-veneris which is acyclic. The walls of the central polar cell are composed of six or more deep lobes. There are two to four other cells surrounding the guard cells. Long axes of the guard cells parallel the veins.

Adaxial epidermal cells present the typical lobed appearance with the long axis of the cells parallel with the adjacent vein. Epidermal cells over the veins are elongated with straight walls.

No hairs, glands, or scales were observed.

Blechnum L.
Plate VIII, Fig. 37

The leaf is hypostomatic. Stomata vary from acyclic to monocyclic. The monocyclic types themselves intergrade to yield examples of both acyclic and monocyclic types.

Cells of the adaxial surface are deeply lobed to give a branched appearance. The long axis of these cells runs parallel to the veins. Epidermal cells over the veins are elongated.

Scattered three to four-celled glands are found attached to the abaxial surface over the veins (Plate IX, Fig. 1).

PLATE VIII

Figure 36. Adiantum capillus-veneris (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 37. Blechnum spicant (A) upper epidermis, (B) lower epidermis.

Figure 38. Woodwardia fimbriata (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

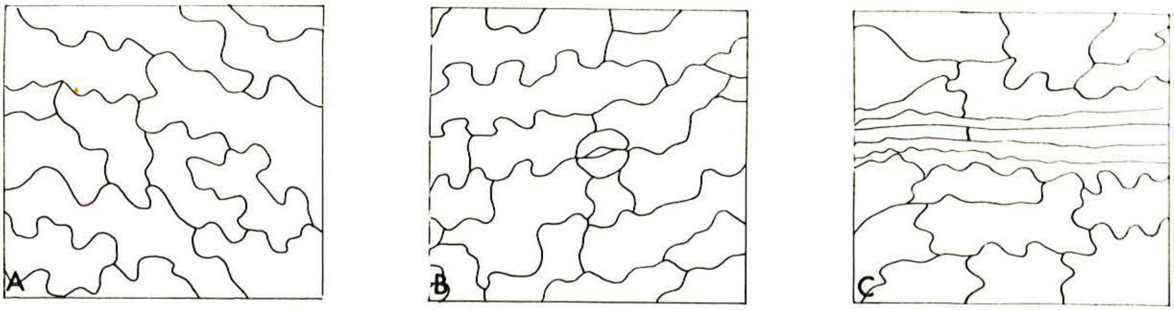
Figure 39. Asplenium vespertinum (A) upper epidermis, (B) lower epidermis.

Figure 40. Polypodium scouleri (A) upper epidermis, (B) lower epidermis.

200 μ

VIII

36



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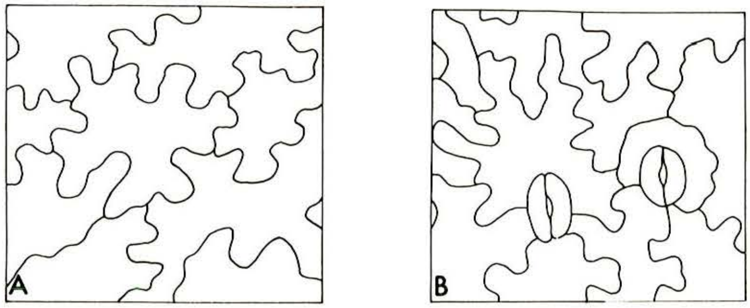

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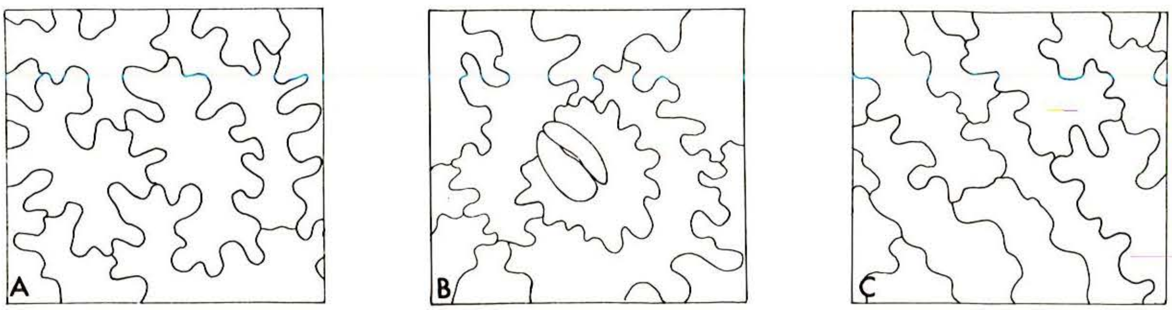

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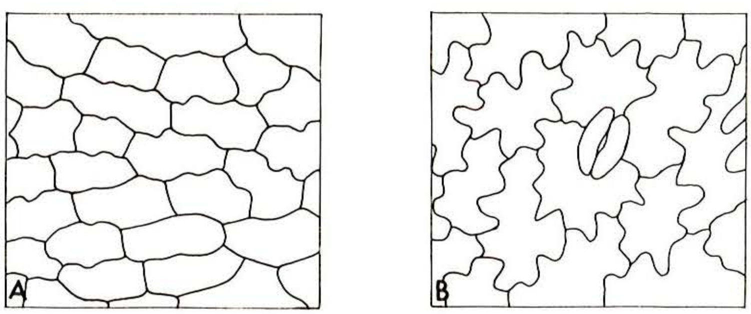

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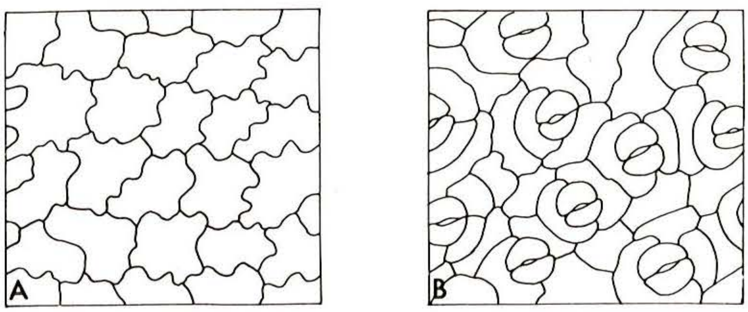


Woodwardia Sm.
Plate VIII, Fig. 38

The leaf is hypostomatic. Stomata are monocyclic; the central polar cell is multilobed. As many as twelve lobes may be present. The other cells surround the remaining portion of the guard cells. The long axis of the guard cells is parallel to the veins.

Abaxial epidermal cells are elongated over the veins. The adaxial epidermis is very similar to Blechnum spicant with its deep, almost branching lobes and irregular outlines.

Asplenium L.
Plate VIII, Fig. 39

The leaves are hypostomatic. The stomata are monocyclic; the lobes of the central polar cell have pointed "teeth." There are seven or more of these "teeth." Two to three other epidermal cells surround the guard cells beside the central polar cell.

The cells between the stomata are also lobed with pointed "teeth" in contrast to the cells on the adaxial surface which are more brick-like in arrangement with some waviness of the cell walls.

There is no differentiation over the veins, adaxial or abaxial. The long axes of the cells run parallel to the adjacent veins.

Polypodium L.
Plate VIII, Fig. 40
Plate IX, Figs. 41, 42

The leaves are hypostomatic. Stomata are monocyclic; the central polar cell is smaller than in the other genera of ferns examined. Cell walls of this cell are markedly less sinuous. This is particularly true of _Polypodium scouleri_ in which no undulations are found in the polar cell walls. The central polar cell caps the two guard cells. The margins of the polar cells of _Polypodium californicum_ and _Polypodium glycyrrhiza_ are more sinuous, but still present a "cap-like" appearance. The remainder of the guard cells is surrounded by two to three epidermal cells.

Adaxial epidermal cells are also sinuous but much more so than the abaxial surface. The adaxial cells of _Polypodium californicum_ are much larger than the adaxial cells of _Polypodium scouleri_ and _Polypodium glycyrrhiza_. The cells are elongated over the veins with few or no undulations.

PLATE IX

Figure 41. Polypodium californicum (A) upper epidermis, (B) lower epidermis, (C) upper epidermis over a vein.

Figure 42. Polypodium glycyrrhiza (A) upper epidermis, (B) lower epidermis, (C) lower epidermis over a vein.

Figure 1. Blechnum spicant, gland from lower epidermis.

Figure 2. Woodwardia fimbriata, rare gland from over the veins.

Figure 3. Pityrogramma triangularis var. viscosa, gland from leaf edge.

Figure 4. Aspidotis californica, gland from lower epidermis.

Figure 5. Cryptogramma acrostichoides, gland from lower epidermis.

Figure 6. Polypodium scouleri, gland from lower epidermis.

Figure 7. Polypodium californicum, gland and lower epidermis.

Figure 8. Polypodium glycyrrhiza, lower epidermis with gland.

IX

41

42

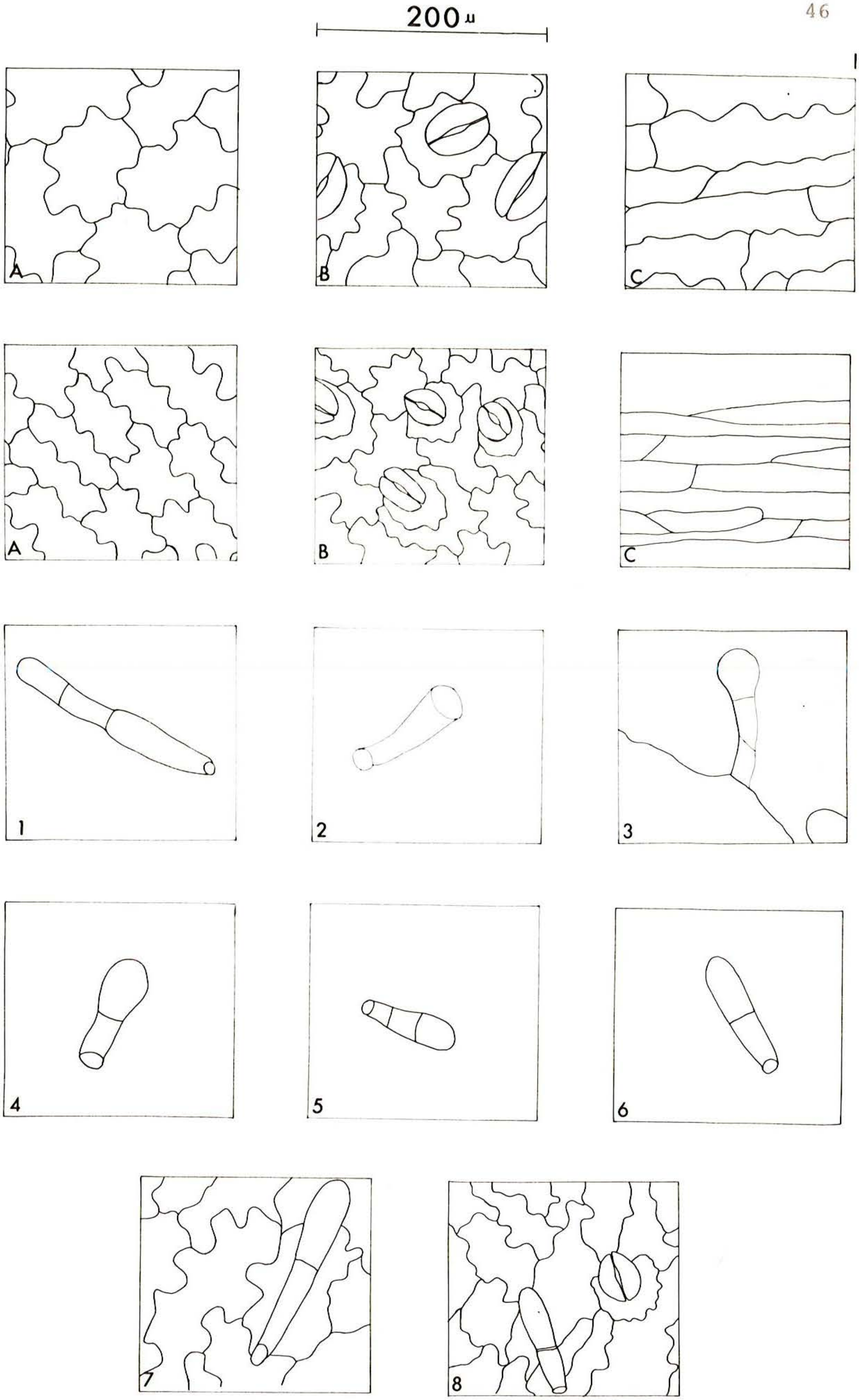


PLATE X

Figure 9. Pteridium aquilinum, hairs.

Figure 10. Cheilanthes feei, hair.

Figure 11. Cheilanthes parryi, hair.

Figure 12. Woodsia scopulina, micro-hair from lower epidermis.

Figure 13. Polystichum dudleyi, scale found over the veins.

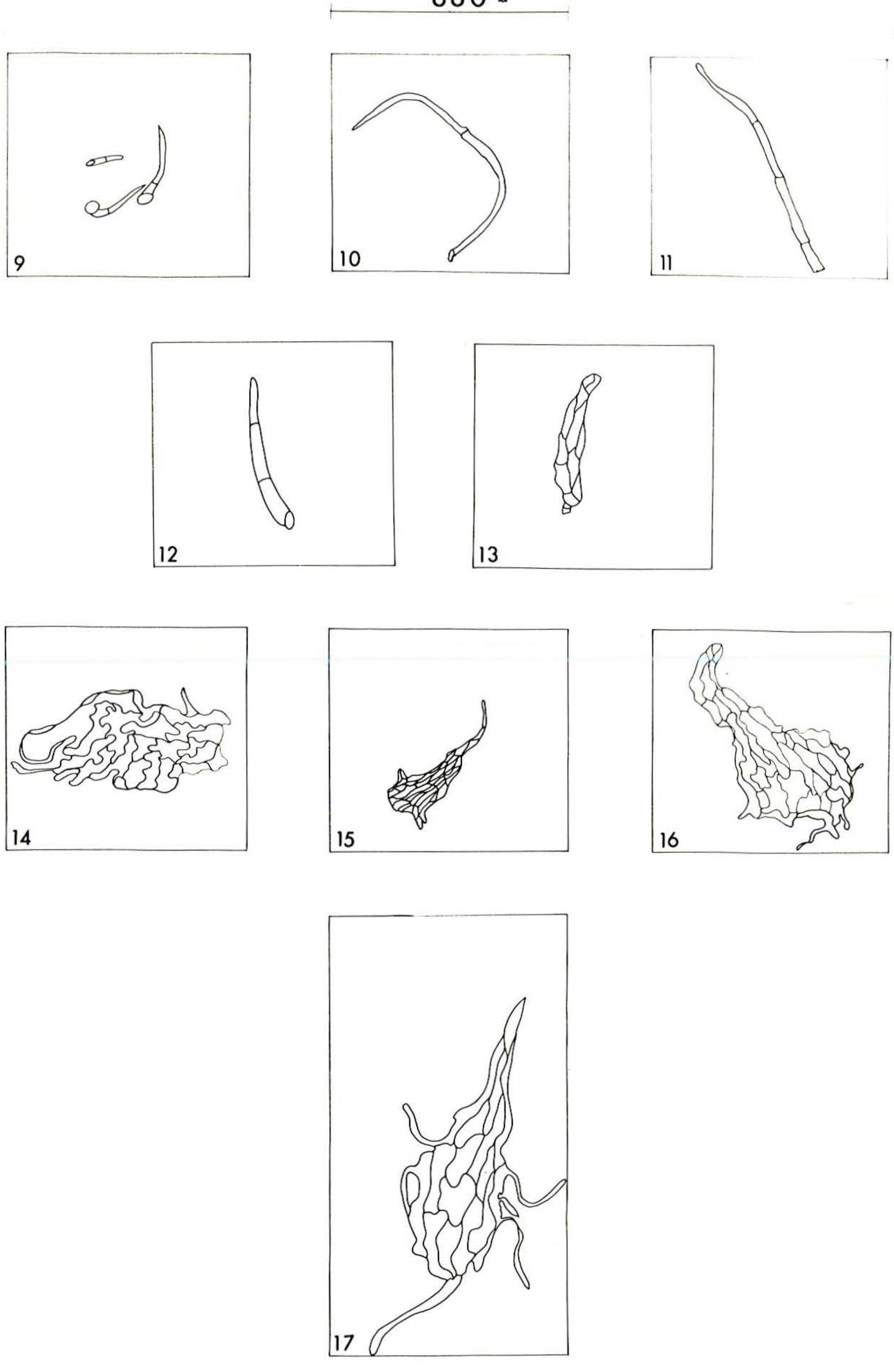
Figure 14. Cheilanthes clevelandii, scale.

Figure 15. Polystichum munitum, scale from lower epidermis.

Figure 16. Cheilanthes covillei, scale from lower epidermis.

Figure 17. Cheilanthes wootonii, scale from lower epidermis.

X



DISCUSSION AND CONCLUSION

The ferns investigated can be placed under the family types of Maróti (1958) by using the four stomata cell classifications. Two of these four cell classifications are represented in California under the three family types. These are:

(A) The Ophioglossaceae type characterized by the amphistomatic leaf; epidermal cells angular, elongated with straight walls; stomata acyclic; the guard cells surrounded by five, six, or seven epidermal cells. Botrychium represents the genus studied in this group.

(B) The Marsileaceae type characterized by a potentially amphistomatic leaf; lower epidermis elongated and irregular; upper epidermis wavy or undulating; stomata acyclic; guard cells surrounded by three epidermal cells. Marsilea represents the genus studied in this group.

(C) The Polypodiaceae type which is characterized by the hypostomatic leaf; undulating radial walls of the epidermal cell; stomata monocyclic; guard cells surrounded by two or three epidermal cells. Intermediates are found which appear to be transitional from the acyclic condition.

On the basis of the observations, the Polypodiaceae type can be divided into three sub-types:

(1) The Polypodium sub-type characterized by undulating radial cell walls except for the walls of the central polar cell which do not undulate or undulate less so than the Athyrium type below. The two genera Aspidotis and Polypodium are included in this sub-type.

(2) The Athyrium sub-type characterized by the fact that the radial walls of the abaxial and abaxial epidermal cells are undulated to form two arms to resemble a "T" or to form deep "V" formations (Plate V, Fig. 24, B; Plate V, Fig. 25, B); deeply undulating walls of the central polar cell; guard cells surrounded

by three to four epidermal cells. *Athyrium*, *Pteridium*, *Woodsia*, *Asplenium*, *Polystichum*, *Dryopteris*, *Woodwardia*, *Cystopteris*, *Pityrogramma*, and *Adiantum* (except for *Adiantum capillus-veneris* which is acyclic) are included in this sub-type.

(3) The *Blechnum* sub-type which appears to be an intermediate between the two preceding sub-types. The cells are acyclic or intermediate between acyclic and monocyclic, but do not have the straight ground epidermal cell walls of the Ophioglossaceae tpe. The genera *Cryptogramma*, *Cheilanthes*, *Blechnum*, *Pellaea*, *Onychium*, and *Adiantum capillus-veneris* are included under this sub-type.

In all ferns studied the long axes of the guard cells and the abaxial and adaxial epidermal cells are parallel to the long axis of the leaf.

Epidermal cells are generally elongated over the veins in the direction of the long axis of the leaf.

The glands or micro-hairs have the same general appearance in all species except for Woodwardia fimbriata. The glands in this species present a "trumpet" appearance (Plate IX, Fig. 2).

Scales are similar except for Polystichum dudleyi (Plate X, Figs. 13) which has a small stalk.

The ferns studied easily fall under the three family types described by Maróti (1958). The large Polypodiaceae family of the older classification is itself variable as is evidenced by the three sub-types into which it may be divided. Munz and Keck (1959) have divided the old family Polypodiaceae into five families, but even these are not constant in epidermal patterns. For example, the nine genera of their family, Pteridaceae, contain monocyclic, acyclic, and intermediate cell types. The families Aspidiaceae, Aspleniaceae, and Polypodiaceae, respectively, contain monocyclic genera, but the family, Blechnaceae, contains one monocyclic type genus and one of intermediate type.

Some genera (Polypodium, Aspidotis, Cystopteris, Pityrogramma, Asplenium, Athyrium, Pteridium, Woodsia, Polystichum, Dryopteris, and Woodwardia) are constant, but others (Cryptogramma, Blechnum, Pellaea, and Onychium) are variable. However, in many of these genera only one species was examined. The most variable genus of all is Cheilanthes which contains acyclic and intermediate types which tend toward the monocyclic condition.

During the course of the study many problems became apparent which could not be included in the scope of this investigation. Chief among these is the question of the origin of the guard cells of the stomata, the central polar cell origin, and the origin of the ground epidermal cells adjacent to the guard cells. One difficulty here seems to be the lack of an appropriate nomenclatural system for the ontogenetic study of these cells. Metcalfe and Chalk (1950) proposed a typology for the mature patterns formed by the stomata and neighboring cells in dicotyledons. Four main types were proposed, but there are variations in these types and some probably merit separate designations. Metcalfe (1960) and others (Stebbins and Kush, 1961) have described four categories of stomatal complexes in the monocotyledons. The types with many subsidiary cells are considered to be derived by reduction in the number of subsidiary cells. Florin (1951) has ably considered the origin of the subsidiary cells in the gymnosperms in which he describes two modes of development. One mode of development is the haplocheilic in which the guard cells originate directly from the primary mother cell of the guard cells. The second mode is the syndetocheilic in which the primary mother cell of the guard cell usually divides into three cells of which the median cell gives rise to the guard cells and the two lateral cells function as subsidiary cells. These are developmental terms, but according to Stace (1965) they should be abandoned because they are now being applied to mature complexes rather than to developmental stages. Stace (1966) felt that it is dangerous to equate mature subsidiary cell arrangements to categories based upon modes of development.

In the author's opinion, a better classification would be to utilize the terms perigene (subsidiary cells not arising from the primary mother cell) and mesogene (subsidiary cells which do arise from the primary mother cell). This type of classification has been combined with the terms haplocheilic and syndetocheilic in the works of Maróti (1961) and Pant and Mehra (1964). The terminology has the advantage of being equally applicable to all groups of plants rather than primarily to the gymnosperms. Developmental studies could provide valuable evidence for a classification of stomata which could prove to be of considerable taxonomic and phylogenetic value. Perhaps the extreme variability of the genera *Cheilanthes* and *Blechnum* could be explained from such studies.

Studies in plant physiology may reveal the factors responsible for the development of a stomatal meristemoid.

Allsopp (1955), working with *Marsilea*, was successful in arresting or inducing the formation of stomata. He did not succeed in achieving any changes in the patterns of their development, however.

Another question which arose during the course of this study is the effect of the environment upon the plant. The position of the leaves upon the plant is also important. In most plants the lower leaves are in a different environment from the upper leaves, and their structure may reflect this. In this work an effort was made to utilize pinnae from the same position upon the plant. However, a study of the epidermis from all portions of the plant could prove valuable.

Variations may be due to three factors: developmental, positional, and environmental. Similarity of environment may have favored similar adaptations in widely different ferns. Before attempting to utilize any character taxonomically, the ecology of ferns should be investigated further.

Distribution and size of the stomata upon the leaf may be of value also. This was particularly noticeable in the fern *Polypodium scouleri* (Plate VIII, Fig. 40, B) which was collected from a seashore environment. Several species should be collected from many different environments to determine what impact the environment may have upon development. Stomatal frequency could be a family, generic, or specific criterion, or of no systematic importance at all. In addition, stomatal size is frequently directly affected by the level of ploidy (Stace, 1965).

Still another question to be answered is what causes epidermal cell walls to undulate. According to Linsbauer (1930), waviness is affected by environmental conditions prevailing during leaf development. Another suggestion is that waviness is due to the method of hardening of the cuticle (Watson, 1942). Studies of this nature could perhaps answer some of the problems of diversity.

Variability of epidermal patterns exhibited by the family Polypodiaceae, as revealed in this study, points to its polyphyletic origin. A thoroughly investigated new character could cause some shifts in the balance of evidence. Any comparison between two closely allied taxa reveals a few characters which are conspicuously different, e.g., the hairs and scales are not a constant feature in the genera *Cheilanthes* and *Polystichum*. This fact does not impair the usefulness of these structures in the floras for identification of these ferns. Epidermal characters are likely to provide a means of identification in sterile material, and they can serve as a valuable tool in taxonomic and phylogenetic considerations.

Previous studies of the sori may turn out to be of more importance in the study of ferns than the study of epidermal characters. The epidermal characters can, however, be regarded as significant in the overall picture of complete systematic evidence.

SUMMARY

1. The epidermis of forty-two species of native ferns of California was investigated with particular reference to the arrangement of the epidermal cells around the stomata.
2. These ferns were found to fall into three types: the Ophioglossaceae, the Marsileaceae, and the Polypodiaceae family types.
3. The Polypodiaceae type can be further subdivided into the _Polypodium_, the _Athyrium_, and the _Blechnum_ generic sub-types.
4. The types are characteristic for the Ophioglossaceae and Marsileaceae families, but the Polypodiaceae family type is quite variable.
5. Variability among genera of the Polypodiaceae is particularly noticeable.
6. It is not possible to separate all species on epidermal cell arrangement.
7. Ontogenetic studies of guard cell and subsidiary cell formation are useful to reveal patterns of development.
8. Studies of stomatal distribution and frequency are recommended.
9. The ecology of ferns should be investigated further to obtain information of the environmental impact upon the plant.

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LITERATURE CITED

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